



β -SECRETASE ENZYME COMPOSITIONS AND METHODS

Field of the Invention

5 The invention relates to the discovery of various active forms of β -secretase, an enzyme that cleaves β -amyloid precursor protein (APP) at one of the two cleavage sites necessary to produce β -amyloid peptide ($A\beta$). The invention also relates to inhibitors of this enzyme, which are considered candidates or leads for therapeutic agents in the treatment of amyloidogenic diseases such as Alzheimer's disease. Further aspects of the present invention include screening methods, assays, and kits for discovering such therapeutic inhibitors, as well as diagnostic methods for determining whether an individual carries a mutant form of the enzyme.

Background of the Invention

15 Alzheimer's disease is characterized by the presence of numerous amyloid plaques and neurofibrillary tangles present in the brain, particularly in those regions of the brain involved in memory and cognition. β -amyloid peptide ($A\beta$) is a 39-43 amino acid peptide that is major component of amyloid plaques and is produced by cleavage of a large protein known as the amyloid precursor protein (APP) at a specific site(s) within the N-terminal region of the protein. Normal processing of APP involves cleavage of the protein at point 16-17 amino acids C-terminal to the N-terminus of the β -AP region, releasing a secreted ectodomain, α -sAPP, thus precluding production of β -AP. Cleavage by a putative β -secretase enzyme of APP between Met⁶⁷¹ and Asp⁶⁷² and subsequent processing at the C-terminal end of APP produces $A\beta$ peptide which is highly implicated in Alzheimer's pathology (Seubert, *et al.*, in *Pharmacological Treatment of Alzheimer's* disease, Wiley-Liss, Inc., pp. 345-366, 1997; Zhao, J., *et al.* J. Biol. Chem. 271: 31407-31411, 1996).

It is not clear whether β -secretase enzyme levels and/or activity are inherently higher in Alzheimer's patients than normal; however, it is clear that the cleavage product of this enzyme, $A\beta$ peptide, is abnormally concentrated in amyloid plaques in the brain of